

Plasticity in Metamorphic Traits of Rice Field Frog (*Rana limnocharis*) Tadpoles: The Interactive Effects of Rearing Temperature and Food Level

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Abstract In organisms with complex life cycles, such as amphibians, morphological variation is strongly influenced by environmental factors (e.g. temperature) and maternal effects (e.g. diet). Although temperature and food level exert a strong influence on larval growth, little is known about the interacting effects of these factors on age and size at metamorphosis. In this study, plasticity in growth rates, survival, larval period, and size at metamorphosis were examined in Rice field Frog (*Rana limnocharis*) under different combinations of rearing temperature and food level. Rearing temperature did not affect age at metamorphosis, but a significant interaction between temperature and food level revealed that of tadpoles feeding at a high food level, those reared at 32°C had a shorter length of larval period than those reared at 29°C or 26°C. Similarly, our results also showed high food level produced a larger growth rate and mass at metamorphosis at 32°C, but not at 29 and 26°C. Therefore, our results revealed that the effects of food level on larval growth and metamorphosis were highly dependent on developmental temperature.

Keywords metamorphosis, *Rana limnocharis*, larval period, phenotypic plasticity, rearing temperature, food level

1. Introduction

In animals with complex life cycles, such as amphibians, metamorphic size and timing of metamorphosis are important fitness components (Arnold and Wassersug, 1978; Wilbur, 1980). Larval amphibians are particularly likely to encounter variation in temperature and resource availability due to variation in aquatic breeding habitats (Morey and Reznick, 2004; Skelly, 2004). In addition to energy uptake, temperature can be considered the most important proximal cause of variation in size and age at metamorphosis (Newman, 1998; reviewed by Álvarez and Nicieza, 2002; Castano *et al.*, 2010). Generally, larval anurans living in cold temperatures

have prolonged developmental periods, but they are also larger as metamorphs than conspecifics living at warmer temperatures. Thus, some previous studies have found that age and size at metamorphosis may be larger in anurans living in cold temperatures (von Bertalanffy, 1960; Atkinson, 1996; Beck and Congdon, 2000; Merila *et al.*, 2000; Álvarez and Nicieza, 2002; Laugen *et al.*, 2003; Palo *et al.*, 2003; Liess *et al.*, 2013). However, in low temperatures, an increase in the time required to process food can set a limit for intake rates, which would reduce the gain associated with the higher energy value of the diet (reviewed by Álvarez and Nicieza, 2002).

Other factors affect metamorphic timing and growth rate, such as food supply, water level, and the type and density of competitors and predators (Rose, 2005). High food availability accelerates growth and developmental rates, thus allowing tadpoles either to maximize size at metamorphosis or minimize the length of the larval phase (Pandian and Marian, 1985; Arendt and Hoang, 2005; Peacor and Pfister, 2006). Contrarily, low resource

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availability due to low food level, high larval density, or both, would also constrain metamorphosis, such that if conditions were poor throughout the larval period, then tadpoles growing at a slow rate might take a long time to reach the minimum size for metamorphosis (viewed by Wilbur and Collins, 1973). However, water temperature has contradictory effects on growth rate and body size such as higher temperatures result in faster growth to a smaller size at metamorphosis. Few studies have examined how temperature and food level can interact to affect amphibian tadpoles (viewed by Castano *et al.*, 2010).

In this study, we examined the effects of food level and temperature on size and age at metamorphosis of the Rice field Frog (*Rana limnocharis*). From mid-May to early September, Rice field frogs breed in small bodies of water, from temporary or semi-permanent ponds to very short-lived rain pools (Fei and Ye, 2001). Consequently, larval viability relies on rapid development; eggs hatch after 3–4 days at 26–30°C (mean temperature = 28°C), which is the temperature range most often observed in the breeding ponds (Wu and Sun, 1981). We tested experimentally how sensitivity to temperature and its interaction with food level has influenced variation both in the length of larval period and in the body size of *R. limnocharis* at metamorphosis.

2. Materials and Methods

2.1 Study species and rearing condition We collected 100 eggs from each of 10 egg masses in one population in Shihe County (32°08' N, 114°01' E, altitude 84 m), Henan, the central plains of China, in early May 2011. Each egg mass was kept in one 100-l plastic container with an automatic aerator, and all hatched on the same day. Tadpoles were at the same developmental stage (Gosner stage 26, absorption of external gills and fully formed spiracle; Gosner, 1960) at the start of the experiment. Room (air) temperature was kept at 26°C. In the experiments where temperature was manipulated, aquarium heaters were used to raise the temperature to 29°C and 32°C. Three temperatures (26°C, 29°C, and 32°C) were chosen because they fall within the range this species experiences in the field or lab (Wu and Sun, 1981; Shi *et al.*, 2012). Tadpoles were exposed to a 12L:12D photoperiod throughout the study period and the water in the containers was changed weekly.

2.2 Experiment design A 2×3 factorial design was used to examine the effects of food level and temperature on larval growth rates and post-metamorphic performance

(Table 1). To evaluate the effects of food level, half of the tadpoles in each temperature treatment were placed on a low food regimen (LF, 6% of per tadpole mass per day) and half were placed on a high food regimen (HF, 12% per tadpole mass per day). Larvae were fed with commercial fish food (Bieyanghong, Biological Co. Ltd., Hangzhou, China, medium protein content, MPC; 30% protein, 10% lipids, 18% algae, 4% fiber, 10% ash). A total of 420 tadpoles were randomly allocated into each of six experimental treatments ($n = 70$). For each temperature, 140 individual vessels (70 for each diet treatment), each of which is 0.3L (diameter = 77 mm), were randomly placed into two rectangular tanks (110 cm × 90 cm × 60 cm; L × W × H) filled with fresh water to a depth of 8 cm.

Table 1 A 2×3 factorial design was used in this study. HT: high temperature, MT: medium temperature, LT: low temperature, HF: high food level, LF: low food level

Temperature (°C)	Food level	
	High	Low
32	HT + HF	HT + LF
29	MT + HF	MT + LF
26	LT + HF	LT + LF

2.3 Data analysis After the first metamorph (defined as the emergence of the first forelimb, stage 42; Gosner, 1960) was discovered, all tadpoles in the six plastic containers were checked daily. All metamorphs found were collected and kept in plastic vials with sand and 1 mm of water until tail re-sorption was completed. Several variables were measured: (1) age at metamorphosis (number of days from the beginning of the experiment until first metamorphosis); (2) mass at metamorphosis (Gosner stage 46; tail re-sorption was completed), weighed with an electric balance to the nearest 0.001 g; (3) growth rate (measured as the mass at complete metamorphosis divided by the age at metamorphosis); and (4) survival rate (the proportion of tadpoles surviving until metamorphosis).

Length of larval period, mass at metamorphosis, survival rate, and growth rate were analyzed with a generalized linear model (GLM) with type III mean squares using temperature and food level treatment as fixed factors. If the overall GLM results were significant, the data were analyzed with ANOVAs by using post hoc multiple comparisons (Fisher's LSD) or a Chi-square test to evaluate differences between food levels or between temperatures (SPSS 13.0, SPSS Inc., 2004, Chicago, IL,

USA). All *P*-values given are two-tailed, with values presented as means \pm standard error.

3. Results

The effects of rearing temperature and food level on length of larval period were not significant (temperature, $F_{2,293} = 2.13$, $P = 0.12$; food level, $F_{1,155} = 2.60$, $P = 0.11$, Table 2, Figure 1), but revealed a significant temperature \times food level interaction ($F_{2,293} = 10.95$, $P = 0.024$). At 32°C, HF tadpoles had shorter larval period lengths than those reared at the 29°C and 26°C temperatures (Tukey's Post hoc tests all $P < 0.035$), but no difference was observed in larval period length between 29°C and 26°C ($P = 0.83$). However, LF tadpoles had similar larval period lengths among the three experimental temperatures (Post Hoc all $P > 0.086$).

Mass at metamorphosis was significantly affected by temperature ($F_{2,293} = 4.05$, $P = 0.018$) and food level ($F_{1,293} = 10.21$, $P = 0.002$). There was a significant interaction between food level and temperature ($F_{2,293} = 3.42$, $P = 0.034$). *Post hoc* tests revealed significant differences between HF tadpoles reared at 32°C and 29°C ($P = 0.004$), and between 32°C and 26°C ($P < 0.001$, Figure 1). The effect of food level differed according to temperature. HF tadpoles were significantly larger than LF tadpoles at 32°C, but not at 29°C ($P = 0.31$) and 26°C ($P = 0.38$).

Although effects of temperature were not significant

($F_{2,293} = 0.64$, $P = 0.53$) on growth rate, food level ($F_{1,293} = 4.41$, $P = 0.037$) and temperature \times food level interaction ($F_{2,293} = 5.82$, $P = 0.003$) did affect growth rate. HF tadpoles had a greater growth rate than LF tadpoles at 32°C ($P < 0.001$), but not at 29°C ($P = 0.34$) or 26°C ($P = 0.20$). HF tadpoles reared at 32°C had a significantly or marginally significantly larger growth rate than those raised at 29°C and 26°C (all $P < 0.059$, Table 1), while there was no difference at 29°C or 26°C (all $P > 0.06$). Similarly, LF tadpoles reared at 29°C had a greater growth rate compared with those at 32°C and 26°C ($P = 0.049$), but not between 32°C and 26°C ($P = 0.86$).

Temperature and food level had a significant effect on survivorship to metamorphosis (all $P < 0.001$, Table 2, Figure 1), but the interaction between rearing temperature and food level was not significant ($F_{2,414} = 1.60$, $P = 0.20$). Tadpoles at 26°C suffered from significantly reduced survival compared to those at 32°C and 29°C ($P < 0.002$), but not between 29°C and 32°C. HF larvae showed higher survival compared to LF larvae at 26°C (Chi-square test: $\chi^2 = 5.31$, $df = 2$, $P = 0.021$), but not to larvae at 29°C ($P = 0.18$) or 32°C ($P = 0.44$).

4. Discussion

In this study, rearing temperature did not affect age at metamorphosis, but a significant interaction between rearing temperature and food level revealed HF tadpoles

Table 2 The generalized linear model for the effects of temperature and food level on metamorphic traits in a *Rana limnocharis* population. T: temperature; F: food level.

Response variable	Source of variation	df	MS	F-value	P-value
Length of larval period	T	2	1249.97	2.13	0.121
	F	1	1526.89	2.60	0.108
	T \times F	2	2210.13	3.77	0.024
	Error	293	587.02		
Body mass	T	2	0.01	4.05	0.018
	F	1	0.01	10.21	0.002
	T \times F	2	0	3.42	0.034
	Error	293	0		
Growth rate	T	2	0.03	0.64	0.529
	F	1	0.24	4.41	0.037
	T \times F	2	0.31	5.82	0.003
	Error	293	0.05		
Survival	T	2	1.52	8.04	< 0.001
	F	1	4.40	23.34	< 0.001
	T \times F	2	0.30	1.60	0.203
	Error	414	0.19		

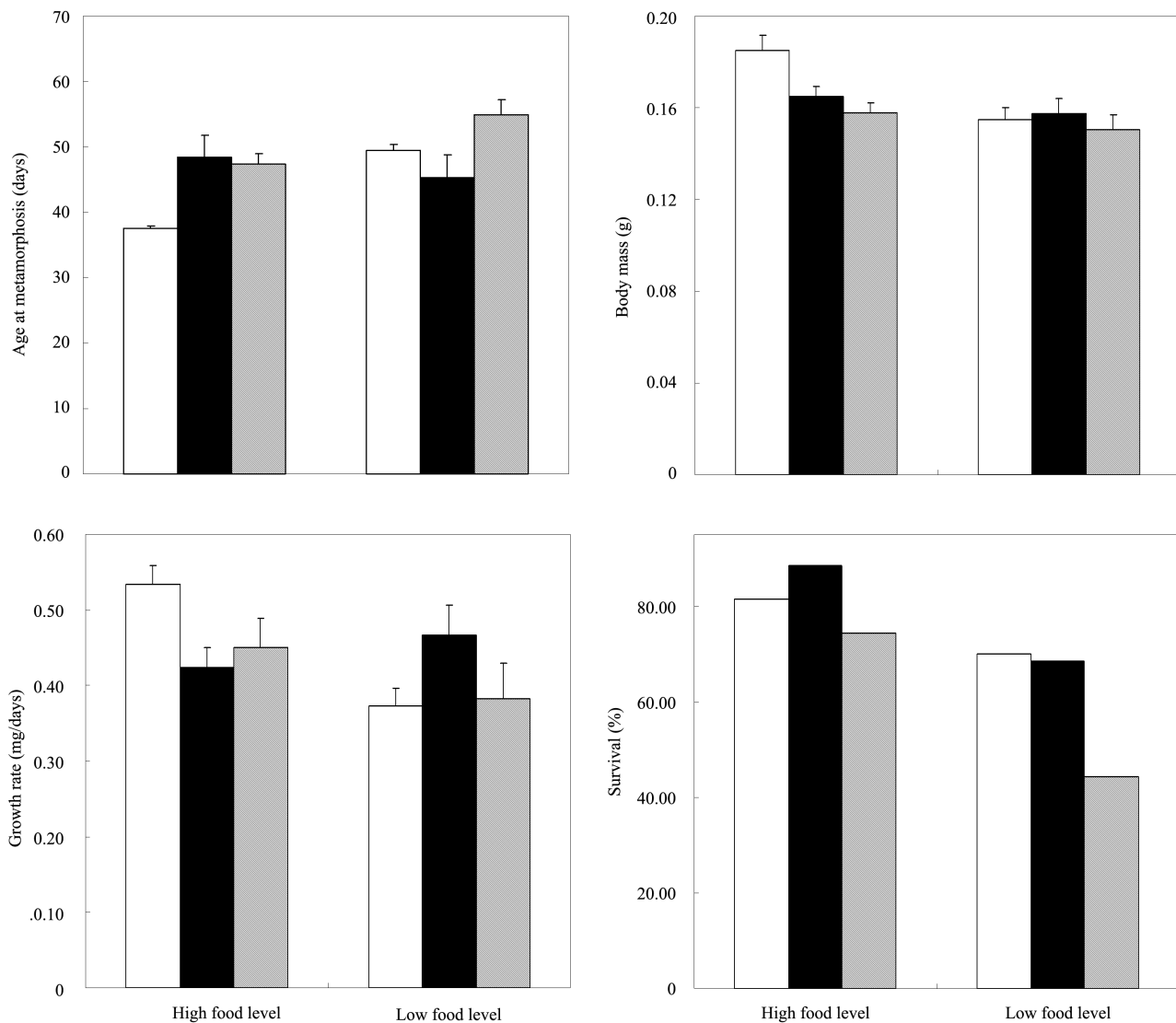


Figure 1 Influences of temperature and food level on age, mass, growth rate, and survival at metamorphosis of *Rana limnocharis* (open columns, 32°C; black columns, 29°C; Gray columns, 26°C).

at 32°C had shorter larval period lengths than those reared at 29°C and 26°C. High food availability would likely cause faster growth, leading to both shorter larval periods and an increase in metamorphic size (Pandian and Marian, 1985), but our results revealed that the effects of food level on larval period were closely dependent on developmental temperature.

Our results also showed food level influenced the growth rate of Rice field frog tadpoles. An interesting finding was the significant interactions between temperature and food level, suggesting that HF tadpoles reared at 32°C had a significantly larger growth rate and mass at metamorphosis than those raised at 29°C and 26°C. This is similar to the pattern found in some

populations of anurans (e.g. Laugen *et al.*, 2005; Buchholz and Hayes, 2000; Castano *et al.*, 2010). Therefore, an important finding of the previous study was that the larvae with higher growth rates tended to have larger body sizes at any point in time including metamorphic climax, and tended reach metamorphosis early (Berven, 1982; viewed by Woodward *et al.*, 1988; Riha and Berven, 1991; Loman, 2002). In addition to growth rates, metabolism may be an underlying mechanism for tadpoles at high food level to have a larger mass at high temperatures. High food availability will permit the elevated metabolic rate associated with higher temperatures (e.g. Arendt and Hoang, 2005; Lindgren and Laurila, 2009), whereas growth rate and metabolism may be more constrained at

cool temperatures and low food levels.

Temperature and food level also influenced survivorship, with more tadpoles surviving at warmer temperatures and high food level. However, there was not a significant interaction between temperature and food level. Our results are in accordance with Sanuy *et al.* (2008) and Orizaola and Laurila (2009), but contrary to Castano *et al.* (2010), who found that survivorship was higher at cooler temperatures in wood frog tadpoles (*Rana sylvatica*). The differences may relate to adaptations for specific temperatures. Rice field frogs begin to breed in early summer and finish in early autumn. Moreover, they prefer to lay eggs in small pools, which may reach higher temperatures during the summer; thus Rice field frog tadpoles may be better adapted to warmer temperatures. Taken together, if the growth condition is good, global climate change or local manipulations of the environment may promote growth and development of Rice field frog tadpoles in natural ponds.

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